

providing a universal primer configured to bind with said first universal primer binding region;

providing an additional primer configured to bind with a second end of said target nucleic acid sequence; and

incubating said first primer pair, universal primer, and additional primer in the presence of said target nucleic acid sequence under conditions that amplify said target nucleic acid.

23. The method of Claim 22, wherein the first stem region and the first target binding region are connected by a flexible linker.

24. The method of Claim 23, wherein the flexible linker is selected from the group consisting of polyethylene glycol, polypropylene glycol, polyethylene, polypropylene, polyamides and polyesters.

25. The method of Claim 22, wherein the second stem region and the second target binding region are connected by a flexible linker.

26. The method of Claim 25, wherein the flexible linker is selected from the group consisting of polyethylene glycol, polypropylene glycol, polyethylene, polypropylene, polyamides and polyesters.

27. The method of Claim 22, wherein the first oligonucleotide primer comprises one or more modified bases.

28. The method of Claim 22, wherein the second oligonucleotide primer comprises one or more modified bases.

29. The method of Claim 22, wherein the target nucleic acid sequence is DNA.

30. The method of Claim 22, wherein the target nucleic acid sequence is RNA.

31. The method of Claim 22, wherein said incubating comprises the Polymerase Chain Reaction.

32. The method of Claim 22, wherein said amplifying is mediated by an enzyme selected from the group consisting of: Taq polymerase and reverse transcriptase.

33. The method of Claim 22, wherein said additional primer comprises:
a third oligonucleotide primer comprising a third target binding region configured to bind to a second end of said target nucleic acid sequence, a reverse transcriptase binding

region, and a third stem region, wherein said third stem region comprises a second universal primer binding region; and

a fourth oligonucleotide primer comprising a fourth target binding region configured to bind to said second end of said target nucleic acid sequence, and a fourth stem region that is homologous with said third stem region, so that said third oligonucleotide primer and said fourth oligonucleotide primer join together to form a second primer pair that binds to said target nucleic acid sequence.

34. A method of amplifying a target nucleic acid sequence, comprising the steps of:
providing a target nucleic acid sequence having a first amplification region and a second amplification region;

selecting from a library of first oligonucleotide primers, a first oligonucleotide primer that comprises a first target binding region that is homologous with a portion of the first amplification region, wherein said first oligonucleotide primer comprises a first stem region and a first universal primer binding region;

selecting from a library of second oligonucleotide primers, a second oligonucleotide primer that comprises a second target binding region that is homologous with a second portion of the first amplification region, wherein said second oligonucleotide primer comprises a second stem region that is homologous with said first stem region, so that said first oligonucleotide primer and said second oligonucleotide primer join together to form a first primer pair that binds to said target nucleic acid sequence at said first amplification region;

providing a universal primer configured to bind with said first universal primer binding region;

providing an additional primer configured to bind with a second amplification region of said target nucleic acid sequence; and

incubating said first primer pair, universal primer, and additional primer in the presence of said target nucleic acid sequence under conditions that amplify said target nucleic acid.

35. The method of Claim 34, wherein the library of first oligonucleotide primers comprises greater than 65,536 oligonucleotides.

36. The method of Claim 34, wherein the first target binding region comprises at least six nucleotides.

37. The method of Claim 34, wherein the second target binding region comprises at least six nucleotides.

38. The method of Claim 34, wherein the first target binding region and the first stem region are connected by a flexible linker.

39. The method of Claim 38, wherein the flexible linker is selected from the group consisting of polyethylene glycol, polypropylene glycol, polyethylene, polypropylene, polyamides and polyesters.

40. The method of Claim 34, wherein the second target binding region and the second stem region are connected by a flexible linker.

41. The method of Claim 40, wherein the flexible linker is selected from the group consisting of polyethylene glycol, polypropylene glycol, polyethylene, polypropylene, polyamides and polyesters.

42. The method of Claim 34, wherein the first oligonucleotide primer comprises one or more modified bases.

43. The method of Claim 34, wherein the second oligonucleotide primer comprises one or more modified bases.

44. The method of Claim 34, wherein the first oligonucleotide primer comprises one or more modified backbone linkages.

45. The method of Claim 34, wherein the second oligonucleotide primer comprises one or more modified backbone linkages.

46. The method of Claim 34, wherein the target nucleic acid sequence is DNA.

47. The method of Claim 34, wherein the target nucleic acid sequence is RNA.

48. The method of Claim 34, wherein said incubating comprises the Polymerase Chain Reaction.

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49. The method of Claim 28, wherein said amplifying is mediated by an enzyme selected from the group consisting of Taq polymerase and reverse transcriptase

Respectfully submitted,

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